Mastozoología Neotropical, 32(1):e01139, Mendoza, 2025
Versión on-line ISSN 1666-0536
https://doi.org/10.31687/saremMN.25.32.01.09.e1139

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Artículo Original

GENETIC CHARACTERIZATION OF WILD PIGS IN A RECENTLY COLONIZED AREA OF THE AGRICULTURAL CORE OF ARGENTINA

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ABSTRACT. In recent years, concerns about wild pigs (*Sus scrofa* Linnaeus, 1758) as an invasive exotic species have increased significantly in Argentina due to their negative impact on ecosystems, agriculture, livestock, and health. The geographical expansion of these invasive wild pigs has occurred through both natural spread and human-mediated activities. This expansion has led to the establishment of new populations, as could be the case in the most productive agricultural region of the country, where wild pigs have been observed for approximately a decade. Given that genetic diversity plays a crucial role in understanding the success of invasion capability, this study provides the first genetic characterization and hybridization assessment between morphotypes of a potential new wild pig population in the core agricultural region of Argentina, using the mitochondrial control region and the NR6A1 gene as molecular markers. Phylogenetic analysis revealed a predominance of European ancestry, with a minority of specimens belonging to the Asian clade, suggesting multiple origins. The individuals studied exhibited notable genetic diversity, surpassing that reported in other wild populations in Argentina and Latin America and similar to the diversity found in native populations in Europe and Asia. In addition, specimens of different morphotypes, including those with domestic ancestry, pure wild boars, and hybrids, were identified. The results emphasize the genetic potential of the studied wild pigs to adapt and successfully expand in newly invaded areas, underscoring the need to implement immediate management and control strategies.

RESUMEN. ESTUDIO GENÉTICO DE CERDOS SILVESTRES EN EL NÚCLEO AGRÍCOLA DE ARGENTINA. En los últimos años, las preocupaciones sobre los cerdos silvestres (*Sus scrofa* Linnaeus, 1758) como especie exótica invasora han aumentado significativamente en Argentina debido a su impacto negativo en los ecosistemas, la agricultura, la ganadería y la salud. La expansión geográfica de los cerdos silvestres invasores ha ocurrido tanto por dispersión natural como por actividades mediadas por el ser humano. Esta expansión ha dado lugar al establecimiento de nuevas poblaciones, como podría ser el caso de la región agrícola más productiva del país, donde los cerdos silvestres han comenzado a ser observados hace aproximadamente una década. Dado que la diversidad genética juega un papel crucial en la comprensión del éxito en la capacidad de invasión, este estudio proporciona la primera caracterización genética y evaluación de hibridación entre morfotipos de una potencial nueva población de cerdos silvestres en la región agrícola central de Argentina, utilizando marcadores moleculares región de control y NR6A1. El análisis filogenético reveló predominancia de ascendencia europea, con una minoría de ejemplares del clado asiático, lo que sugiere una multiplicidad de orígenes. Los individuos estudiados mostraron una notable diversidad genética, que supera la reportada en otras poblaciones silvestres en Argentina y América Latina, y similar a la observada en su distribución nativa

Recibido 20 agosto 2024. Aceptado 11 novoembre 2024. Editora asociada: Cecilia Lanzone

en Europa y Asia. Además, se identificaron ejemplares de distintos morfotipos, que incluyen aquellos con ascendencia doméstica y jabalíes puros, así como híbridos. Los resultados enfatizan el potencial genético de los cerdos silvestres estudiados para adaptarse y expandirse con éxito en áreas recientemente invadidas, lo cual subraya la necesidad de implementar estrategias de manejo y control inmediatas.

Key words: Genetic diversity, Hybridization, Invasive species, Pampa ecoregion, *Sus scrofa*.

Palabras clave: Diversidad Genética, Especie Invasora, Ecorregión pampa, Hibridación, *Sus scrofa*.

Cite as: Yolanda A. Gloazzo Gimenez, Diana B. Acosta, Carlos E. Figueroa, Matías E. Mac Allister, Gabriela P. Fernández, & Mariano L. Merino. 2025. Genetic characterization of wild pigs in a recently colonized area of the agricultural core of Argentina. Mastozoología Neotropical, 32(1):e01139. https://saremMN.25.32.01.09.e1139

INTRODUCTION

Sus scrofa (Linnaeus 1758), native to Eurasia and North Africa, is now present on all continents except Antarctica and numerous oceanic islands, making it one of the most widely distributed mammals globally (Barrios-Garcia & Ballari 2012). This species is one of the first mammals intentionally introduced by humans for meat outside its native range (Courchamp et al. 2003; Barrios-Garcia & Ballari 2012). More recent introductions, including those in Argentina, have been driven by commercial hunting (Courchamp et al. 2003; Barrios-Garcia & Ballari 2012).

In Argentina, wild populations of *S. scrofa* comprise two general morphotypes: wild boars and feral pigs (Fig. S1). Feral pigs are descendants of domestic pigs introduced by Spanish colonizers in the 16th century along the coast of Río de la Plata in Buenos Aires, where they released the animals upon abandoning their settlement (Acosta et al. 2019; Figueroa et al. 2022). These wild pigs rapidly dispersed across southeastern Buenos Aires Province and the northern region of Río Negro Province (Schmidl 1903; Iriart 1997; Acosta et al. 2019; Figueroa et al. 2022). European wild boars were introduced for hunting in La Pampa in the early 20th century, with specimens imported from France. From this initial introduction, the boars were translocated by humans to Río Negro, Entre Ríos, and even to Uruguay (Daciuk 1978; Amieva 1993; Figueroa et al. 2022). Another introductory event involving wild boars occurred in the first decade of the 20th century when animals imported from Spain were released on Victoria Island in the province of Neuquén (Biedma 2003; Figueroa et al. 2022). Since then, repeated introductions of both feral pigs and European wild boars have occurred, including both intentional and unintentional releases and movements. As a result, the two wild morphotypes have expanded their range through two independent processes: natural dispersal and human-mediated translocations, currently occupying 12 of the 18 ecoregions of Argentina (Ballari et al. 2019). This significant range expansion has resulted in contact between the two wild morphotypes, allowing for potential breeding and hybridization areas (Figueroa et al. 2022). Consequently, it has become almost impossible to distinguish between morphotypes in many areas. In addition, several scientific studies have reported that hybridization may enhance the adaptive potential of the species and increase its invasiveness (Iacolina et al. 2018; Scandura et al. 2022).

Sus scrofa is regarded as one of the most significant invasive alien species (IAS) in Argentina, classified as Category A due to its highest level of risk and impact (Lizarralde 2016). It poses major threats to biodiversity, ecosystem integrity, agriculture, fisheries, and public health (Barrios-Garcia & Ballari 2012; Lizarralde 2016). The success of IAS is attributed to intrinsic species factors such as reproductive rate, body mass, abundance, and natural distribution area, and extrinsic habitat factors, which include the availability of vacant niches, food resources, climate, and absence of predators (Barrios-Garcia & Ballari 2012). Among these factors, genetic variability is crucial for understanding the invasive potential of the species, as it could enhance adaptability to new environments, increased resistance to diseases, and greater ability to colonize and establish in new areas (Lawson Handley et al. 2011).

Genetic studies in Argentina have focused on wild pig populations, both wild boars and feral pigs. These studies primarily utilize molecular characterization of neutral markers and phylogenetic analysis at introduction sites (Sagua et al. 2018; Acosta et al. 2019, 2023; Figueroa et al. 2022). A more comprehensive study revealed high genetic diversity compared to specimens in their native range and suggested a pre-

dominantly European origin, with some exhibiting Asiatic ancestry due to crossbreeding with improved domestic breeds (Acosta et al. 2019, 2021; Figueroa et al. 2022). Additionally, the degree of hybridization in feral populations suggests a potentially greater adaptive capacity, due to increased introgression of allelic variants (resulting in higher genetic diversity) and enhanced phenotypic plasticity (Fontanesi et al. 2014; Iacolina et al. 2018).

Despite these findings, significant information gaps persist, particularly in the most productive agricultural region of Argentina, encompassing the provinces of Buenos Aires, Santa Fe, and La Pampa. This area is crucial for both crop and livestock production, especially pork. Backyard farming is prevalent, with small-scale operations focused on self-consumption and the sale of byproducts. According to the 2018 National Agricultural Census, more than 65% of agricultural operations raise pigs primarily for personal use, while the remainder is dedicated to commercial meat production, with a small fraction (1%) aimed at genetic improvement (INDEC 2021). Although potential distribution studies for *S. scrofa* in Argentina suggest that this region should not support the presence of such animals, it has indeed become an area of recent wild pig colonization (La Sala et al. 2023; Ballari et al. 2024). These wild pigs are relatively new to the area, with the first sightings documented around ten years ago (Gloazzo Gimenez 2024). These pigs have negatively impacted crops, reducing corn yields through cob consumption, trampling, breaking silage bags, and damaging soil composition through rooting (Duboscq-Carra et al. 2021; Marcos et al. 2021). Moreover, many wild pigs have been captured for breeding by local farmers, and some have escaped back into the wild, increasing crossbreeding among species strains (Gloazzo Gimenez 2024).

Therefore, due to the significant lack of genetic information and understanding regarding the origins of these specimens, this research aims to provide the first comprehensive genetic characterization and assessment of hybridization in a potentially novel wild pig population within the core agricultural region of Argentina. This study is crucial for the development of effective management strategies to tackle the challenges posed by wild pigs in agricultural environments.

MATERIALS AND METHODS

Study area and sample collection

The study area is situated in the Pampas ecoregion, Argentina, encompassing the provinces of Buenos Aires, Santa Fe, and La Pampa (**Fig**. 1). The Pampas ecoregion constitutes the most important grassland ecosystem in Argentina (Matteucci 2012). It is characterized by a prairie ecosystem and a temperate sub-humid climate, with average annual temperatures between 14 and 20 °C and rainfall ranging from 700 to 1200 mm/year (Matteucci 2012). The predominant vegetation was a grass steppe or pseudo-steppe, with floodable plains comprised of brackish substrates and halophilic vegetation (Matteucci 2012). However, the Pampas ecoregion is the oldest agricultural-livestock production area in Argentina, which has led to significant landscape loss due to the disappearance of the Pampas grasslands, natural habitats, and biodiversity caused by land use changes since the colonial era. What was once a matrix of natural grassland with patches of crops has transformed into a matrix of crops from which patches of natural grassland have gradually disappeared (Matteucci 2012). Currently, the area known as the "agrarian triangle of Argentina" represents a subarea within the Pampas ecoregion and is recognized as the core agricultural zone of the country, renowned for being the most fertile region in the nation, with the study encompassing a considerable extent of this sector. Approximately 90% of its land surface is dedicated to agricultural activities, primarily the cultivation of corn, soybeans, and sunflowers. Additionally, a significant portion is dedicated to cattle and pig farming (Ministerio de Agricultura, Ganadería y Pesca 2017).

Between 2018 and 2021, 45 samples of epithelial and muscular tissues were collected from wild pigs at eleven geographic locations (**Fig**. 1; Table S1; Fig. S1). All samples were obtained from authorized sources, including hunters and collaborators, who adhered to the guidelines established by the American Society of Mammalogists (ASM) for the ethical and sustainable use of wild animals in research. These guidelines emphasize sustainable harvesting, ethical hunting practices, and habitat conservation, ensuring that all animals are captured and sacrificed in a manner that minimizes suffering and respects wildlife laws (Sikes 2016). It is important to note that, as previously mentioned in the introduction, we will refer to the specimens analyzed in this study as wild pigs, as it is not possible to determine by morphology whether they are wild boars or feral pigs. Tissue samples were stored in 96% ethyl alcohol at -20 °C in the sample bank of the Centro de Bioinvestigaciones (Pergamino, Argentina) until processing.

Laboratory and data analysis

Genomic DNA was extracted following the phenol-chloroform protocol (Barnett & Larson 2012). Depending on the amount of pellet observed, the DNA was eluted in 50-100 μL of Tris-EDTA buffer and stored at -20 °C. The extraction efficiency, as well as the quality of the genomic DNA, were evaluated using a 1% w/v agarose gel stained with ethidium bromide (10 mg/mL) and visualized under UV light.

For genetic characterization, two molecular markers were employed: a mitochondrial neutral marker, the control region (CR), and a nuclear marker, the Nuclear Receptor Subfamily 6 Group A Member 1 (NR6A1) gene.

The CR marker was chosen primarily for its high polymorphism, which has been observed in most mammal species surveyed to date as demonstrated in extensive literature. Furthermore, this marker enables the calculation of genetic diversity indices and the investigation of the

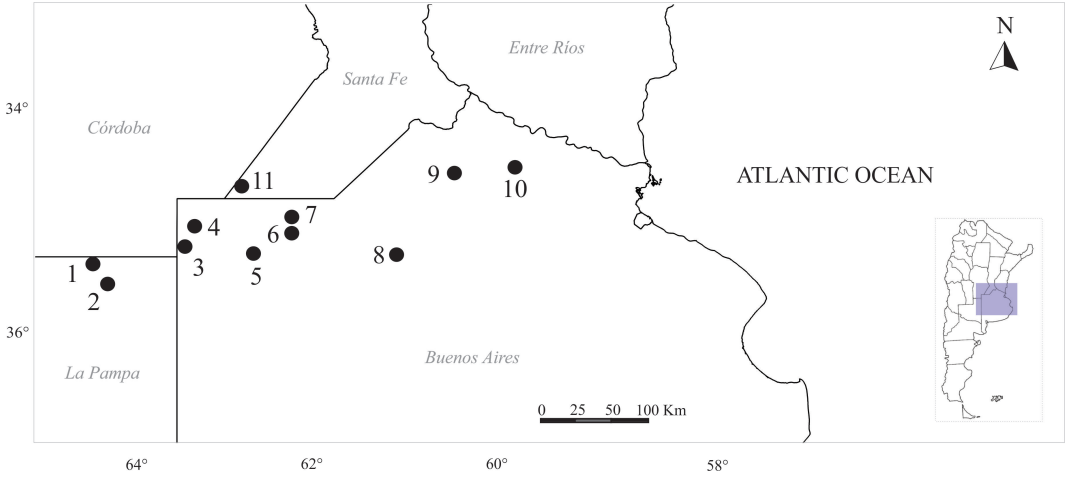

Fig. 1. Wild pigs sampling sites within a recently colonized area in the core agricultural region of Argentina. The numbers indicate the 11 sampling sites in the provinces of Buenos Aires, Santa Fe, and La Pampa. 1) Realicó; 2) Alta Italia, Realicó; 3) Santa Regina, General Villegas; 4) Santa Regina, General Villegas; 5) Pichincha, General Villegas; 6) Coronel Granada, General Pinto; 7) Germania, General Pinto; 8) General Viamonte, General Viamonte; 9) Cuenca Río Arrecifes, Capitán Sarmiento; 10) Taquini, Capitán Sarmiento; 11) Rufino, General López.

evolutionary history of species (Alves et al. 2003; Larson et al. 2005, 2010; Kusza et al. 2014). This marker allows the identification of four phylogenetic groups: two European clades, E1 (widely distributed across Europe) and E2 (restricted to the Italian peninsula, Sardinia, and Croatia); an Asian clade (A), which includes domestic pig breeds from China and Japan; and a Near Eastern clade (NE), which includes pigs from Turkey, Syria, Lebanon, Israel, Jordan, Iraq, and Iran. Furthermore, the E1 clade is subdivided into E1-A (Italy, France, Germany, and Austria) and E1-C (Spain, Portugal, Poland, and Hungary) (Alves et al. 2003; Larson et al. 2005, 2010; Kusza et al. 2014).

For the CR marker, a 734 bp fragment between sites 15.390 and 16.124 was amplified by PCR using the primers implemented by Kusza et al. (2014). The reaction was set to a final volume of 20 μL, containing 25-100 ng of template DNA, 1.5 mM MgCl2, 0.2 μM of each primer, 0.2 mM of each dNTP, 1X reaction buffer, 0.5 U of Taq T-Plus DNA polymerase, and ultrapure sterile water to reach the final volume. The thermocycling conditions were 94 °C for five min, followed by 30 cycles of 94 °C for 45 s, 61 °C for 45 s, and 74 °C for one min, with a final extension of 74 °C for ten min. All amplifications were performed in conjunction with a negative control (distilled water). DNA fragment amplification was confirmed by electrophoresis on a 1.5% w/v agarose gel, stained with ethidium bromide (10 mg/mL) and visualized under UV light. Lastly, to quantify the DNA concentration, high-resolution photographs of the agarose gel were taken using GeneSys V1.4.6.0 software (Syngene) and subsequently analyzed with ImageJ software (Abràmoff et al. 2004). The PCR products were purified and sequenced by Macrogen® (South Korea).

The obtained sequences were visualized and manually edited using BioEdit version 7.2 (https://bioedit.software.informer.com/). Haplotype and nucleotide diversities, as well as the number of polymorphic sites, were calculated using DnaSP 6.12.03 (Rozas et al. 2017). Haplotype sequences were uploaded to the nucleic acid sequence database GenBank (http://www.ncbi.nlm.nih.gov/genbank) under accession numbers PQ381297 - PQ381321.

For the phylogenetic analyses, the sequences generated in this study were combined with sequences from the GenBank nucleic acid database (N = 102), using *Phacochoerus aethiopicus* as the outgroup in the phylogenetic tree (Table S3). A multiple alignment was performed for the complete set using the ClustalW algorithm in MEGA version 11 software (Tamura et al. 2021). The alignment was checked and manually corrected. The mutational model that best fits the dataset was determined through JModelTest software v2.1.4 (Darriba et al. 2012).

Phylogenetic trees were constructed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. In the ML phylogeny, node confidence was assessed by bootstrapping with 10.000 replicates (Felsenstein 1985). In the case of BI, the data were converted into BEAST XML format through BEAUti 2.6.3 (Bouckaert et al. 2014). For the tree reconstruction, the following settings were used: a strict clock as the molecular clock rate variation model and a 100.000.000 generation Monte Carlo Markov Chain length, sampling every 1000. All calculations were performed in BEAST2 (Bouckaert et al. 2014). The first 25% of the sampling trees and estimated parameters were discarded as burn-in with TreeAnnotator v2.6.3 (Bouckaert et al. 2014). FigTree v1.4.0 was employed to visualize the phylogenetic tree (Rambaut 2012). Haplotype relationships, as well as relationships between haplotypes from different sampling sites, based exclusively on the haplotypes obtained in this study, were determined using the Median-Joining algorithm implemented in POPART v1.7 (Leigh & Bryant 2015).

Lastly, to evaluate population expansion through the CR marker, neutrality was tested with Tajima's D and Fu's FS tests in Arlequin 3.5 (Excoffier & Lischer 2010).

The NR6A1 marker encodes the number of vertebrae in *S. scrofa* (Mikawa et al. 2007). Wild boars typically possess 19 thoracic and lumbar vertebrae, whereas domestic pigs have 21-23 vertebrae (King & Roberts 1960). Wild boars carry the wild-type allele (C), while domestic pigs are fixed for the mutated allele (T) (Mikawa et al. 2007; Rubin et al. 2012). Consequently, these polymorphisms can be potentially useful for distinguishing wild boars from domestic pigs and their hybrids. Furthermore, according to Fontanesi et al. (2014) and Canu et al. (2016), incorporating additional genes, such as NR6A1 in conjunction with CR, enhances the resolution and accuracy of hybridization assessments among the morphotypes of *Sus scrofa*.

For the NR6A1 marker, the PCR-RFLP technique was used for the detection of polymorphisms. For the PCR, a 203 bp fragment described by Fontanesi et al. (2014) was amplified. The reaction was set to a final volume of 25 μL, containing 100 ng of template DNA, 2 mM MgCl2, 0.2 μM of each primer, 0.2 mM of each dNTP, 1X reaction buffer, 0.5 U of Taq T-Plus DNA polymerase, and ultrapure sterile water to reach the final volume. The thermocycling conditions were 94 °C for 7 min, 30 cycles at 94 °C for 30 s, 61 °C for 30 s, 74 °C for 30 s, and a final extension at 74 °C for five min. All amplifications were performed in conjunction with a negative control (distilled water). DNA fragment amplification was confirmed by electrophoresis on a 2% w/v agarose gel, stained with ethidium bromide (10 mg/mL), and visualized under UV light.

Subsequently, enzymatic digestion for the RFLP was performed in a final volume of 30 μL, including 10 μL (0.1-0.5 μg of DNA) of the PCR product, 1 μL of the MspI enzyme (CutSmart, New England BioLabs), 3 μL of NE 10X buffer, and ultrapure water to reach the final volume. The samples were incubated at 37 °C for 60 min, and the digestion products were observed on a 2% w/v agarose gel, stained with ethidium bromide (10 mg/mL), and visualized under UV light. The polymorphism of the NR6A1 gene g.299084751C>T or p.Pro192Leu can be visualized by the cutting of the wild-type allele (g.299084751C = 180 + 23 bp) by the MspI enzyme, whereas if the mutant allele is present, it is not cut (g.299084751T = 203 bp) (Fontanesi et al. 2014). Based on these results, allele and genotype frequencies were calculated for the *locus*.

RESULTS

A 734 bp fragment of the CR molecular marker was amplified by PCR in N = 42 samples (see Table S1). After cleaning the sequences, we retained a total of 654 bp. The total set of sequences was grouped into 25 haplotypes, containing 48 variable sites (Table S2). The haplotype diversity was Hd = 0.908 ± 0.00112 and the nucleotide diversity was π = 0.01025 ± 0.00200.

To compare with sequences from GenBank, the fragment size was reduced to 584 bp. The full set of *S. scrofa* sequences (N = 143) was grouped into 106 haplotypes. By reducing the number of base pairs to allow efficient comparison, the haplotypes from this study were found in haplotypes H1 to H22 (Table S3). Of these, 19 haplotypes are reported for the first time, while the remaining three (H1, H3, and H15) were previously identified as European wild boars in both their native range (e.g., Iberian Peninsula, Portugal, and UK) and their distribution as an alien species in Argentina, in the provinces of Buenos Aires, Corrientes, La Rioja, and Entre Ríos (Acosta et al. 2019; Figueroa et al. 2022).

Regarding the phylogenetic analysis, both methodologies (BI and ML) showed the same topology (**Fig**. 2). It reveals a clear division into subclades E1-A, E1-C, E2, NE, and A, previously reported for *S. scrofa*. The mutational model that best fits the data was HKY+I+G (Hasegawa et al. 1985). The wild pigs in this study were found within the European subclade E1-A (80.5%) and the Asian subclade A (19.5%) (see Table S3).

The haplotype network obtained using the Median-Joining algorithm for the 25 haplotypes from this study shows that the haplotypes group into two main clades, E1-A and A. Additionally, two central haplotypes (H1 and H16) are positioned in a star-like structure, which may correspond to ancestral haplotypes from which other haplotypes may have diverged through one or two mutations (**Fig**. 3). Furthermore, these two haplotypes are present across multiple sampling sites. Regarding the haplotypes identified by the sampling site, it can be observed that points 2, 5, and 11, corresponding to the three provinces included in this study, displayed the highest diversity, with the greatest number of haplotypes (**Fig**. 4).

The statistical neutrality tests, Tajima's D and Fu's FS, produced negative values of -2.07 and -2.15, respectively. However, the p-value (0.1) was the same for both tests and did not meet the threshold for statistical significance (α = 0.05).

The NR6A1 molecular marker was successfully genotyped for all the specimens analyzed in this study (N = 45). The following genotypic frequencies were found $f_{(TT)}$ = 0.5, $f_{(TC)}$ = 0.17, and $f_{(CC)}$ = 0.33, and allelic frequencies $f_{(T)}$ = 0.585 and $f_{(C)}$ = 0.415. The genotypes for each specimen are detailed in Table S1, while **Figure** 5 presents the genotypes observed at each sampling site. Although there is a higher proportion of T alleles, the nearly equal frequencies of both alleles (T and C) in the studied wild pigs indicate significant diversity. This diversity reflects the possible presence of wild specimens with domestic, wild boar, and hybrid ancestry.

DISCUSSION

In recent years, concerns about wild pigs as an invasive exotic species have grown exponentially in Argentina (Cuevas 2023). These animals have

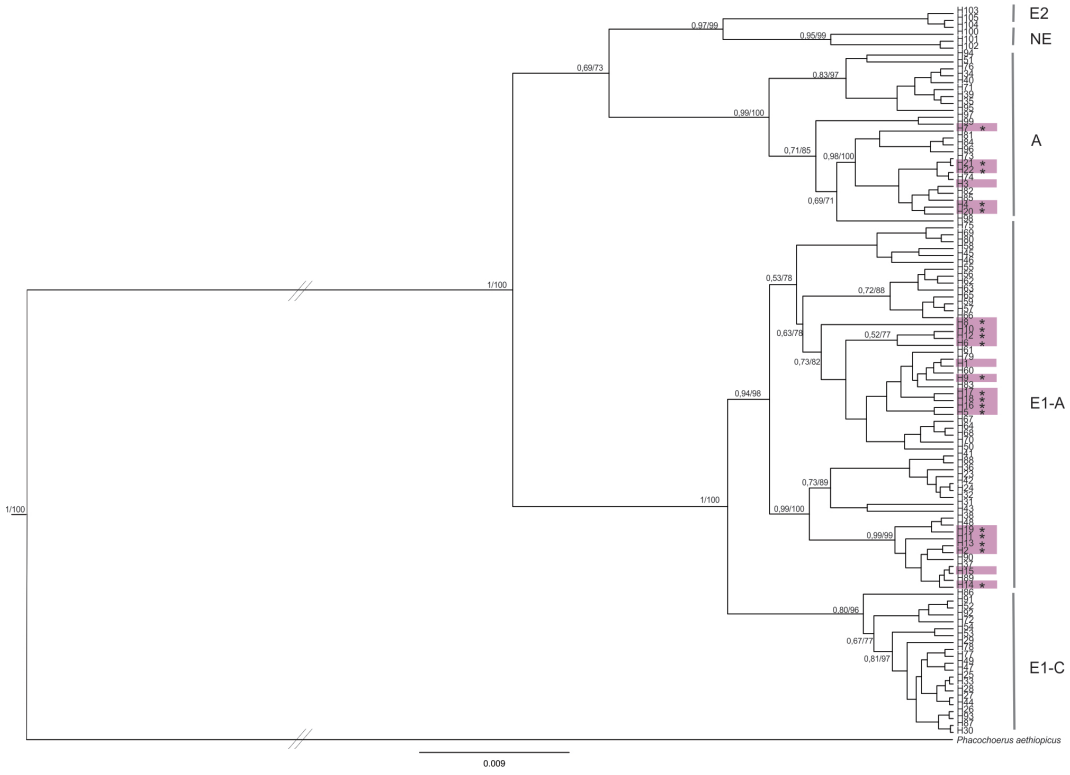

Fig. 2. Bayesian phylogenetic tree obtained for 106 haplotypes based on a 584 bp fragment of the CR molecular marker in *Sus scrofa*. Haplotypes from wild pigs identified in this study are highlighted in purple, and the asterisks within them denote novel haplotypes. Posterior probabilities > 0.50 and bootstrap values > 50 are indicated at the nodes. Clades corresponding to the previously reported haplogroups, such as E1A (Italy, France, Germany, and Austria), E1C (Spain, Portugal, Poland, and Hungary), E2 (Italian Peninsula, Sardinia, and Croatia), NE (Turkey, Syria, Lebanon, Israel, Jordan, Iraq, and Iran), and A (China and Japan), are shown in brackets (Kusza et al. 2014; Scandura et al. 2011).

been shown to cause significant ecosystem disturbances and negatively impact agriculture and livestock (Duboscq-Carra et al. 2021; Marcos et al. 2021). Conversely, wild pig populations are important to small-scale farmers, who regard them as a valuable genetic resource (Carpinetti et al. 2016; Acosta et al. 2021). These populations retain significant genetic diversity compared to elite breeds and possess desirable rusticity traits for adaptation to diverse habitats. For this reason, this study offers the first genetic characterization of a recently emerging wild pig population in the most productive agricultural region of Argentina. This knowledge could advance our understanding of the effectiveness of wild pigs as an invasive exotic species in recently colonized areas.

The phylogenetic analysis strongly supports historical records, with most specimens exhibiting European ancestry (E1-A) and a smaller proportion belonging to the Asian clade (A). Historically, the European wild boar was introduced to La Pampa in the early twentieth century, originating from France (Navas 1987). Therefore, based on the phylogenetic tree results, where over 80% of specimens fall within the E1-A subclade, we can infer that the wild pigs in the study area likely originate from Italy, France, Germany, and Austria, supporting Navas's (1987) claims (see **Fig**. 2; Table S1; S3). Additionally, 19 haplotypes are reported here for the first time, providing new genetic insights beyond previous findings. This new genetic information is significant as it correlates with the natural and human-mediated dispersal of wild boars across several provinces in Argentina, including Santa Fe, Buenos Aires, Córdoba, Neuquén, San Luis, and Río Negro. The distribution of these haplotypes, as shown in the haplotype network (see **Fig**. 3), as well as in the figure of haplotype distribution by sampling sites (see **Fig**. 4), reflects

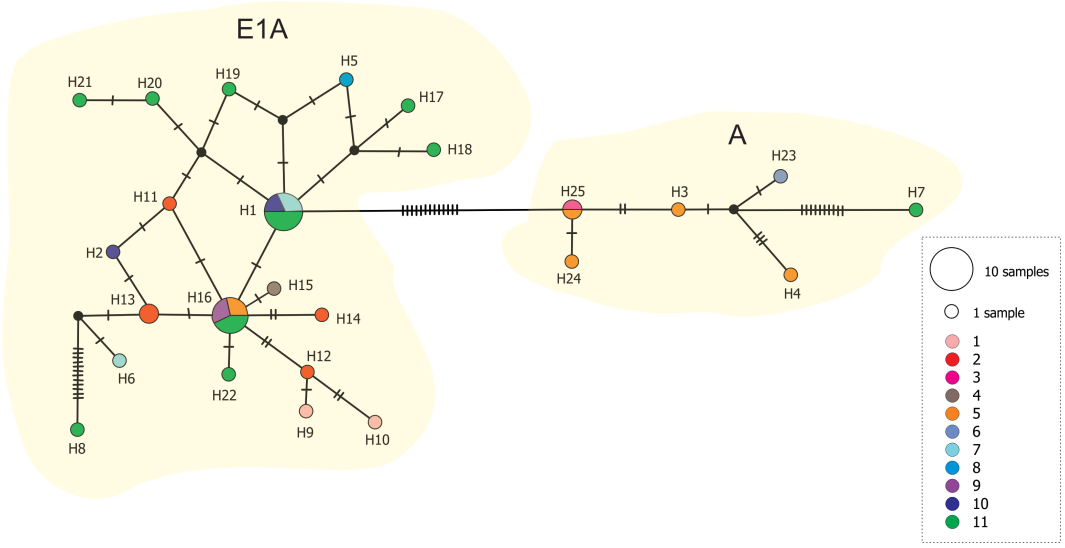

Fig. 3. Median-joining network of the 25 haplotypes obtained from 654 bp of the CR molecular marker in 42 wild pig samples analyzed in this study. The colors represent the sampling sites (1 to 11). Circle size is proportional to haplotype frequencies, and the transverse lines between haplotypes represent the number of mutations separating them.

this geographic spread, with specific haplotypes found across multiple provinces, indicating their movement and establishment throughout the country. This pattern aligns with historical data on the escape and spread of wild boars in the central region of Argentina (Navas 1987; Sagua et al. 2018; Figueroa et al. 2022), further strengthening the correlation between the genetic data and the documented dispersal routes. As for the specimens found in clade A, their presence may result from hybridization with local domestic breeds (Scandura et al. 2008, 2011; Acosta et al. 2021, 2023; Figueroa et al. 2022), or may indicate that the European wild boars introduced to Argentina carried some level of Asian genetic introgression, as reported in other studies; or a combination of both scenarios (Giuffra et al. 2000; Kim et al. 2002; Alves et al. 2003).

Two more frequent haplotypes (Hap 1 and Hap 15; Table S3) have been identified, corresponding to sequences reported from countries within their native distribution as well as from this study, suggesting that these haplotypes could be foundational and involved in the initial colonization of the area, from which other variants subsequently emerged (Alves et al. 2003; Larson et al. 2005, 2010; Fang & Andersson 2006). In relation to the haplotypes identified in this study and those previously reported for Argentina, the sequences correspond to haplotypes found in the provinces of Buenos Aires, Corrientes, La Rioja, and Entre Ríos (see Table S3; Acosta et al. 2019; Figueroa et al. 2022). Additionally, they are more closely related to haplotypes reported in populations from the central regions of the country, such as La Pampa (see Table S3; Sagua et al. 2018; Figueroa et al. 2022). These findings suggest the possibility of multiple origins for the specimens contributing to this potential new population.

Regarding the haplotypic network of the 25 haplotypes identified in this study, a star-like pattern is observed, centered on haplotypes 1 and 16, which may suggest that these haplotypes are ancestral or foundational (see **Fig**. 3; Table S3). Although the neutrality tests, Tajima's D and Fu's FS, based exclusively on the specimens from this study, were not significant, they showed a pronounced negative trend, indicating an excess of low-frequency haplotypes consistent with recent population expansion (Ramos-Onsins & Rozas 2002), thereby supporting the results of the haplotype network.

The genetic diversity observed in this recently formed population, as measured through haplotypic and nucleotide diversity of the molecular marker CR (Hd = 0.908 ± 0.00112), is higher than that reported in previous studies for wild populations of the species in other regions where it exists as an exotic species, both in Argentina (Hd = 0.827 ± 0.017: Sagua et al. 2018; Hd = 0.710 ± 0.033: Acosta et al. 2019; Hd = 0.738 ± 0.031: Acosta et al. 2023; Hd = 0.893

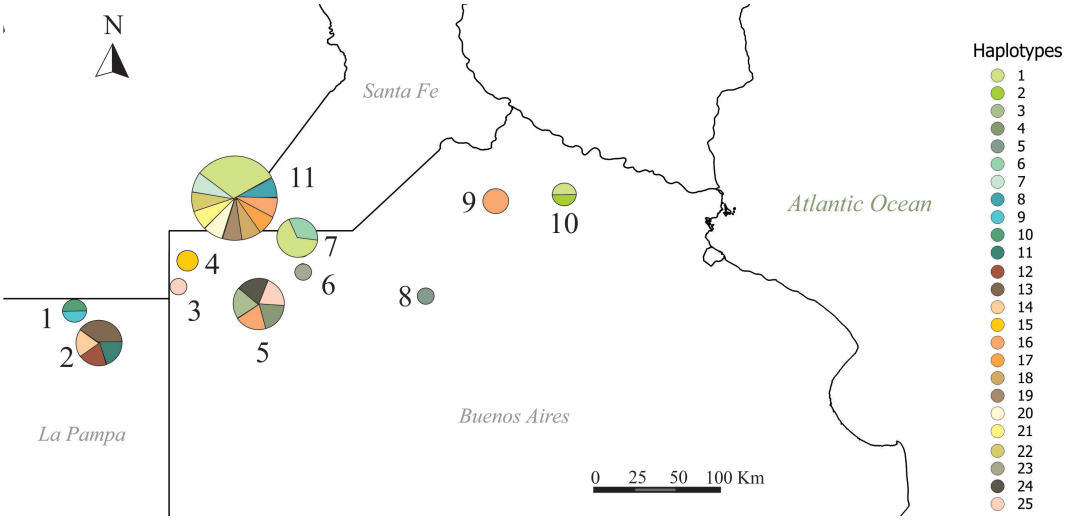

Fig. 4. Distribution of the 25 haplotypes in the CR molecular marker across the 11 sampling sites in the core agricultural region of Argentina. The haplotypes are shown in color, while the circle sizes correspond to their frequencies.

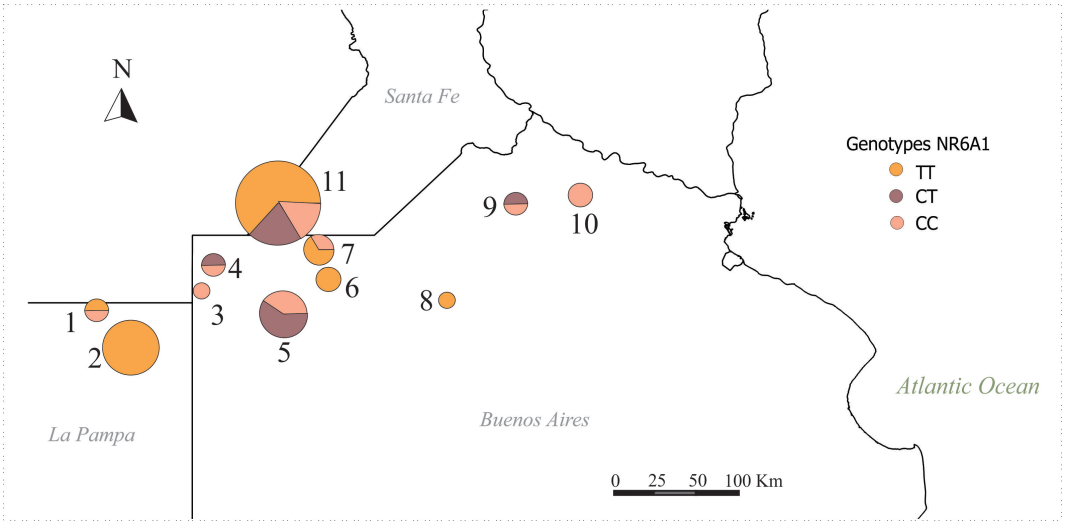

Fig. 5. Distribution of NR6A1 genotypes across the 11 sampling sites in the core agricultural region of Argentina. The different genotypes are shown in color, while the sizes correspond to their frequencies.

± 0.012: Figueroa et al. 2022) and in other Latin American countries, such as Brazil (Porco Monteiro del Pantanal, Hd = 0.016 ± 0.016; Porco Monteiro de Brasilia, Hd = 0.333 ± 0.215; and locally adapted pigs, Hd = 0.693 ± 0.693: Silva et al. 2020) and in Chile, a single haplotype was found, preventing the calculation of haplotypic diversity (Aravena et al. 2015). Notably, the genetic diversity found in this study is greater than that observed in the coastal region of Buenos Aires, where significant mixing among the three morphotypes of the species—feral pigs, wild boars, and domestic pigs-is known to occur and where higher diversity indices would be expected. This area has been home to established populations for over 500 years, further supporting the expectation of increased genetic diversity (Acosta et al. 2019, 2021, 2023). Additionally, the diversity indices in this study exceed those found for the same molecular marker in domestic pigs sampled from the same region (Hd = 0.840 ± 0.031: Figueroa

et al. 2024), reflecting the higher genetic diversity of the wild pigs studied. Furthermore, the genetic diversity indices reported here are similar to those documented for the species in its native distribution across Europe, the Iberian Peninsula, and Asia (e.g., Hd = 0.902: Scandura et al. 2008; Hd = 0.93: Alves et al. 2010; 0.912 ± 0.007 van Asch et al. 2012; 0.714 ± 0.023: Kusza et al. 2014). Therefore, considering the limited number of wild pig specimens introduced into the country over a relatively short historical timeframe-domestic pigs that became feral less than 500 years ago and wild boars introduced approximately 100 years ago-low genetic diversity would be expected due to the founder effect experienced by this species in the invaded areas of Argentina. This reduced diversity would be even more anticipated in the specimens analyzed in this study, given the small number of individuals and their recent appearance (less than ten years ago) in the agricultural core of the country, compared to other more established populations. However, the high levels of genetic diversity observed in this study are particularly noteworthy.

On the other hand, the accurate detection of hybridization among *S. scrofa* morphotypes is crucial for understanding invasion success (Iacolina et al. 2018; Scandura et al. 2022). While morphological techniques can differentiate morphotypes, they lack precision and may miss rare mutation effects (Giacometti et al. 2004; Mallet 2005; Šprem et al. 2011, 2014). Therefore, molecular methods are essential for the effective identification of hybridization (Mallet 2005; Šprem et al. 2011, 2014; Clark et al. 2017).

Hybridization events among *S. scrofa* morphotypes have been previously documented using the neutral molecular marker CR (Giuffra et al. 2000; Scandura et al. 2008, 2011; Frantz et al. 2013; Kusza et al. 2014). In our study, we identified three CR haplotypes shared with wild boars, of which only one is found within its native distribution (H15), while the other two occur in Argentina (H1 and H3) (see Table S3; Alves et al. 2003; Larson et al. 2005; Figueroa et al. 2022). Thus, we can assert that only one haplotype is definitively shared with pure wild boar. According to Fontanesi et al. (2014) and Canu et al. (2016), incorporating additional genes, such as NR6A1 alongside CR, enhances the resolution and accuracy of results; thus, we employed both markers in this study.

We found hybrid specimens, as well as both "pure" wild boars and domestic pigs, within the studied population in similar proportions, suggesting a potential multiplicity of origins for the wild pigs in this area (see Table S1). Furthermore, residents in the study area confirmed the movement of "pure" wild boars from La Pampa to the region, as well as their subsequent release into the wild (Gloazzo Gimenez 2024). The considerable diversity of morphotypes in the area is further supported by the varied morphologies observed among specimens, ranging from smaller individuals with straighter skulls, resembling pure wild boars, to larger specimens exceeding 300 kg with significantly curved skulls (see Fig. S1; Rosell et al. 2001). Moreover, the frequency of the wild boar allele C identified in this study ($f_{(C)} = 0.415$) surpasses the previously reported frequency ($f_{(C)} = 0.155$) calculated by Acosta et al. (2023) in the coastal region of Buenos Aires, suggesting a higher proportion of wild boar ancestry within the population.

In contrast, in the native distribution sites of the species, where it is expected that all wild boar specimens would be CC, a smaller proportion of CT individuals have been found (Fontanesi et al. 2014; Ribani et al. 2019). Recent studies in Europe have warned that the genetic integrity of wild boars may be threatened by the presence of alleles from domestic pigs (Goedbloed et al. 2013; Ribani et al. 2019). This indicates that native populations of Eurasian wild boars have experienced introgression from the domestic gene pool, where heterozygous individuals are present, albeit in low proportions (Koutsogiannouli et al. 2010; Frantz et al. 2013; Goedbloed et al. 2013; Fontanesi et al. 2014; Canu et al. 2016; Ribani et al. 2019). These data suggest a bidirectional introgression of wild and domestic alleles in both lineages, as reported by Ribani et al. (2019), whether mediated by humans or occurring naturally, given their overlapping habitats. Such exchanges lead to genomic modifications in *S. scrofa*, where domestic pigs undergo "de-domestication" by incorporating characteristics of wild boars into their genomes, while wild boars are "domesticated" through the reverse process.

The process of de-domestication in domestic pigs offers several benefits to the population involved. Firstly, it increases genetic variability by introducing new variants or alleles. Secondly, improved adaptation of their descendants to wild environments has been observed, positively impacting wild pigs with domestic ancestry (feral pigs) in natural conditions (Waithman et al. 1999; Iacolina et al. 2018; Ribani et al. 2019). This can be explained by the increase in hybrid vigor in response to greater heterozygosity within the population, which also enhances the survival rate until reproductive age and subsequent reproduction (McCann et al. 2014; Iacolina et al. 2018). Lastly, given

the considerable phenotypic diversity and coloration of feral pigs, the introgression of wild boar alleles could aid in the camouflage of these animals in the wild (Iacolina et al. 2018).

Our findings on hybridization and genetic diversity underscore the species' remarkable ability to adapt, survive, and expand in newly invaded areas. This success can be attributed to the genetic and morphological advantages conferred upon the wild pig population, as discussed herein. Additionally, the considerable population expansion observed in wild pigs could be attributed to anthropogenic activities, particularly the unregulated translocation of these animals across the country for economic purposes, primarily hunting. This phenomenon may also be associated with the substantial genetic variability detected in the study area, as well as the potential multiple origins identified, further underscoring the complex dynamics involved.

Studies in Argentina have shown a significant increase in research on exotic species due to their negative impact on ecosystems, agriculture, livestock, and human health (Barrios-Garcia & Ballari 2012; Ballari et al. 2019, 2024; Cuevas 2023). Consequently, understanding the impact of this species is essential for developing effective preservation strategies for both natural and human-altered ecosystems (Cuevas 2023; Ballari et al. 2024). In Argentina, current control measures are limited to certain protected areas (Ballari & Barrios-García 2014), which are insufficient given the extensive distribution of the species across the country. Therefore, it is crucial to implement a strategic project involving regional, provincial, and national institutions to effectively mitigate the impact of this species. Additionally, raising awareness among residents about this species and promoting its sustainable utilization can encourage responsible practices, helping to address the challenges posed by this invasive species.

ACKNOWLEDGMENTS

We thank Nicolas Fillipini, Dario Carabajal, Ariel Corsico, Adrián Ibarra, Cristian Allende, and Saul Vallejos for their help in collecting wild pig samples. Universidad Nacional del Noroeste de la Provincia de Buenos Aires (UNNOBA), Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) provided financial support for the present research. Finally, we want to give a special thanks to the reviewers and editor of the work.

FUNDING

This research was funded by FONCYT: PICTO 2019-00010 and PICT INVI 2021-00299 and, Universidad Nacional del Noroeste de Buenos Aires, PIP number EXP-0679/2022.

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ONLINE SUPPLEMENTARY MATERIAL

Table S1 - **Table** S2 - **Table** S3 - **Figure** S1